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European perspective on human polyomavirus infection, replication and disease in solid organ transplantation

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Abstract

Human polyomaviruses (HPyVs) are a growing challenge in immunocompromised patients in view of the increasing number of now 12 HPyV species and their diverse disease potential. Currently, histological evidence of disease is available for BKPyV causing nephropathy and haemorrhagic cystitis, JCPyV causing progressive multifocal leukoencephalopathy and occasionally nephropathy, MCPyV causing Merkel cell carcinoma and TSPyV causing *trichodysplasia spinulosa*, the last two being proliferative skin diseases. Here, the current role of HPyV in solid organ transplantation (SOT) was reviewed and recommendations regarding screening, monitoring and intervention were made. Pre-transplant screening of SOT donor or recipient for serostatus or active replication is currently not recommended for any HPyV. Post-transplant, however, regular clinical search for skin lesions, including those associated with MCPyV or TSPyV, is recommended in all SOT recipients. Also, regular screening for BKPyV replication (e.g. by plasma viral load) is recommended in kidney transplant recipients. For SOT patients with probable or proven HPyV disease, reducing immunosuppression should be considered to permit regaining of immune control. Antivirals would be desirable for treating proven HPyV disease, but are solely considered as adjunct local treatment of *trichodysplasia spinulosa*, whereas surgical resection and chemotherapy are key in Merkel cell carcinoma. Overall, the quality of the clinical evidence and the strength of most recommendations are presently limited, but are expected to improve in the coming years.

Keywords: Merkel cell carcinoma, nephropathy, polyoma, progressive multifocal leukoencephalopathy, PyVAN, solid organ transplantation, *trichodysplasia spinulosa*, virus
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Hot Topics

- Pre-transplant testing of all SOT donors and all SOT recipients for HPyV-specific antibody, T-cells or DNA in urine, blood or other clinical specimens is not recommended, because current data are not sufficient for guiding organ allocation, risk stratification, immunosuppressive

therapy, screening modalities or prophylactic, preemptive or therapeutic intervention pre- or post-transplant (***BIII***).

- For SOT recipients listed for kidney transplantation after terminal renal failure due to PyVAN in native or transplanted kidneys, testing of plasma BKPyV should be considered and should be undetectable prior to (re-) transplantation or *at least* have declined by >2 $\log_{10}$ GEq/mL compared with documented peak plasma loads (***BIII***).
- All SOT recipients should have an annual skin and lip examination by a qualified health care professional with experience in diagnosing proliferative and malignant skin diseases to identify HPyV-associated skin diseases as well as melanoma and non-melanoma skin cancers (***BII***).
- Post-transplant routine *screening* of SOT recipients for any HPyV DNA (including JCPyV, MCPyV, TSpyV, KIPyV, WUPyV) in urine, blood or other clinical specimens is not recommended (with the exception of BKPyV in kidney transplant recipients), because current data are not sufficient for risk stratification, or for guiding immunosuppressive therapy, screening modalities, or prophylactic, preemptive or therapeutic intervention (***BIII***).
- All kidney transplant recipients should be regularly screened for BKPyV replication in urine (viruria) or plasma (viraemia) to identify patients at increased risk of PyVAN (***AII***).
- In kidney recipients with confirmed (sustained) plasma BKPyV loads or presumptive or proven PyVAN, maintenance immunosuppression should promptly be reduced in a step-wise fashion unless other competing risks are imminent (***AII***).

Introduction

Human polyomavirus (HPyV) species currently encompass 12 members of the genus *polyomavirus* within the family of *polyomaviridae* [1]. The first HPyVs were JC polyomavirus (JCPyV) and BK polyomavirus (BKPyV), both named after the initials of the patients from whom they were first isolated: JCPyV was detected in brain tissue from a patient with progressive multifocal leukoencephalopathy (PML) [2,3] while BKPyV was detected in urine from a kidney transplant patient with ureteric stenosis shedding 'decoy cells' [4,5]. In the past decade, 10 additional HPyVs have been identified by different molecular genetic techniques: the Karolinska Institute (KI-)PyV and Washington University (WU-)PyV in respiratory secretions of patients with unidentified cause of pneumonia [6], the MCPyV in Merkel cell carcinoma and TSPyV in *Trichodysplasia spinulosa*, two skin diseases in chronically immunocompromised patients [7,8], as well as six additional HPyVs, the clinical role of which still needs to be elucidated [9]. Seroprevalence studies indicate that HPyVs infect 30–90% of the general population and are transmitted independently of one another [7–11]. So far, the mode of transmission has not been resolved for any HPyV. However, HPyVs are frequently detectable in different body sites and fluids of healthy immunocompetent individuals, including skin, hair follicles, saliva, urine, faeces and respiratory secretions, and can be found in human sewage [9,11–13]. Thus, these hardy, non-enveloped viruses of 40- to 45-nm diameter are likely to be transmitted by direct person-to-person contact and by exposure to contaminated surfaces, foods and water.

Uncovering the respective route of transmission is hampered by the fact that characteristic clinical manifestations of primary infections have not been identified for any HPyV, presumably because of a mostly subclinical course or because of a clinically unspecific, for example flu-like, presentation. In rare cases, primary HPyV infections have been discussed as the cause of disease manifestations in the central nervous system (CNS), and in the respiratory, renourinary or gastrointestinal tract. However, supporting data (e.g. demonstrating seroconversion) are typically lacking. Evidence of biopsy-proven HPyV disease is largely confined to immunocompromised patients such as transplant recipients. Here, we review the role of HPyV infection, replication and disease (Table 1) in solid organ transplantation (SOT) patients and provide recommendations regarding the pre-transplant and post-transplant screening, and treatment and prevention using the Infectious Diseases Society of America – United States Public Health Service Grading System [14].

Diagnostic Aspects

Nucleic acid amplification testing (NAT) (e.g. by polymerase chain reaction) is the key diagnostic tool to detect HPyV

TABLE 1. Working definitions of virus infection, replication and disease in transplant patients

Virus infection – evidence of virus exposure
– by detecting specific immune responses (virus-specific antibody or T-cells)
or
– by detecting specific viral antigens, nucleic acids
Note: latent infection or low-level replication is difficult to distinguish for persisting viruses (e.g. polyoma-, herpes-, papilloma-, adenoviruses)
Virus replication – evidence of viral replication by at least one of the following
– increasing viral loads
– direct virion antigen detection
– virus isolation by culture
Note: Virus replication without compatible symptoms and signs of disease may be presymptomatic (e.g. require preemptive treatment).
Probable virus disease – evidence of viral replication above clinically relevant thresholds, or together with compatible symptoms and signs of viral syndrome or organ disease, but without histological confirmation
Note: A major contribution of other aetiologies should be excluded.
Proven virus disease – evidence of virus replication plus corresponding specific histopathology

infection and replication with high sensitivity and specificity in different specimens [3,5–9]. Because of the widespread detectability of HPyVs, the clinical significance of qualitative NAT results is difficult to interpret [15]. Instead, quantitative NAT assays can provide a (semi-)quantitative evaluation of the viral burden by reporting viral genome loads expressed as genome equivalents (GEq) per mL of reference specimen volume or per number of diploid host cells [15,16]. However, significant changes in viral genome load must be greater than the variation coefficient of the method, which varies for different analytes and matrices, and also for different assays and even viral load levels [17,18]. As a first approximation, it is commonly accepted that significant differences in viral genome loads in fluids such as plasma or cerebrospinal fluid must be >1 $\log_{10}$ GEq/mL and >2 $\log_{10}$ GEq/mL in urine samples [19]. For tissue samples, the viral genome loads may depend on the amount of tissue and their cell content, which can be expressed as viral loads per number of diploid host cells using host cell reference genes or per total DNA content where 150 000 cells correspond to approximately 1 μg DNA. Because usually not all cells in a tissue sample are infected, tissue viral loads are probably less robust than viral loads in fluid samples. Because of the limited inter-laboratory agreement and the current lack of an internationally validated calibrator for standardization [20], the comparison of serial patient samples is only considered informative when tested by the same assay, preferably in the same laboratory.

Urine cytology is used in some kidney transplant (KT) centres to identify patients shedding 'decoy cells'. Decoy cells are a marker of very high urine viral loads, typically ≥7 $\log_{10}$ GEq/mL as the result of high-level replication in the renourinary compartment, but do not distinguish between BKPyV and JCPyV) [21], or may be confused with effects from other viruses such as adeno- or cytomegalovirus (CMV) [22]. Urine cytology is inexpensive and requires little infrastructure, but considerable pre-analytical logistics in the clinics and analytical laboratory expertise. Prolonged shedding of >2 months and the presence of tubular casts, inflammatory cells in the urinary sediment, and the detection of three-dimensional PyV aggregates by electron microscopy possibly increases the specificity for significant renal disease in KT recipients [23]. However, they have not been examined in larger comparative studies and their role in screening and preemptive management is presently undefined [15].

Antibody testing has been initially performed for JCPyV and BKPyV by haemagglutination inhibition or virus-neutralization. Currently, however, the most widely used assays are based on recombinant expression of the major capsid protein VP1, or parts of the HPyV T-antigens in baculovirus, yeast, *E. coli* or diverse non-human and human cell lines. Purified virus-like particles (VLPs), VP1-fusion proteins or pseudoviruses then permit testing for IgG, IgM and IgA activities by ELISA or testing for neutralization, respectively. There are few data regarding the role of HPyV serology in SOT, with the exception of BKPyV. Most KT recipients have been found to be BKPyV IgG seropositive, but nevertheless may develop high-level viruria, viraemia and nephropathy [24]. This indicates that BKPyV-specific antibodies cannot prevent progression to disease in KT patients and suggests that virus-specific T cells are the key to controlling BKPyV replication. However, transplantation of kidneys from donors with high BKPyV-specific antibody titres into recipients with low or undetectable antibody titres has been associated with an increased risk of BKPyV viruria and viraemia in KT recipients [25–28]. Also, increasing antibody responses, which neutralize specific BKPyV serotypes, have been detected in KT patients post-transplant, suggesting that exposure to new BKPyV serotypes occurs post-transplant in KT patients [29]. These data, together with the identification of the same BKPyV variants in recipients of kidney pairs from the same donors, suggest that BKPyV in the transplanted kidney allograft is a relevant, but not the exclusive, source of BKPyV replication and polyomavirus-associated nephropathy (PyVAN).

Virus-specific T-cell responses to HPyVs have been studied in peripheral blood of SOT patients, mostly for BKPyV [30–37], and to JCPyV, MCPyV and TSPyV in other patient groups [38–44]. Typically, functional assays are used in quantifying the number of cytokine-producing T-cells after stimulation with specific viral epitopes using enzyme-linked immunospot assay or intracellular cytokine staining and flow cytometry. Research data for BKPyV and similar tests for CMV suggest that abundance of virus-specific T cells in the peripheral blood is associated with protection from viral replication and disease (i.e. showing a good positive predictive value in patients with stable maintenance immunosuppression) [45,46]. Moreover, cessation of BKPyV replication was shown to correlate with reconstitution of BKPyV-specific T-cell immunity, whereas ongoing and recurrent BKPyV viraemia was associated with weak or undetectable T-cell responses [33,34,37,47]. Together, the data suggest that BKPyV-specific T cells have an important role in controlling BKPyV replication. However, these BKPyV-specific assays are currently limited by poor negative predictive values (of <50% for subsequent virus replication and/or disease) even when using the more sensitive *in vitro* expansion assay.

HPyV Diseases in SOT Recipients

The key HPyV diseases in SOT are PyVAN because of its high frequency and detrimental course for the renal allograft in KT

recipients [5], and PML because of its high morbidity and mortality [3].

PyVAN is rare in non-kidney SOT, but causes premature renal failure in 1–14% of KT patients, typically in the first 2 years post-transplant [15]. Without intervention, more than 90% of KT patients with PyVAN show a decline in kidney allograft function, which is followed by graft loss in at least 50% of cases. Immunosuppression is a major risk factor for BKPyV replication and development of PyVAN [48–50]. The risk for BK-PyVAN is increased when lymphocyte-depleting antibodies or higher cumulative corticosteroid exposure are used for induction or rejection therapy. A higher risk has also been reported for tacrolimus-mycophenolic acid combinations compared with cyclosporine-mycophenolic acid, higher tacrolimus or mycophenolate exposure or mTOR inhibitor-combinations in large retrospective studies, registry analyses and in prospective randomized trials [51–54]. Further PyVAN determinants are related to the recipient (male gender, older age, low or undetectable BKPyV antibody; low or absent BKPyV-specific T cell responses), the donor (recent BKPyV exposure, HLA mismatch, deceased donation) and the transplant (ischaemia/reperfusion injury, ureteric stents, acute rejection, retransplantation after graft loss due to PyVAN). However, PyVAN incidence rates and risk factors vary between different transplant programmes, and are likely to reflect local differences in organ types and immunosuppression protocols. Other relevant BKPyV-associated diseases include PyV-associated haemorrhagic cystitis, ureteric stenosis, pneumonia, encephalitis and retinitis, all of which are rare in SOT [5]. Rarely, JCPyV may also cause PyVAN in KT recipients who have typically no evidence of relevant BKPyV replication [3,21,55]. Importantly, JCPyV viraemia is much lower or undetectable in JC-PyVAN, impeding its use as a convenient surrogate marker as established for BK-PyVAN high-level viruria, leaving only high urine JCPyV loads as an index of suspicion. [21].

Progressive multifocal leukoencephalopathy is caused by JCPyV and has been reported in practically all SOTs, including KT, heart transplant (HT), lung transplant (LuT), liver transplant (LiT) and kidney-pancreas transplant (KPT) recipients, showing a high mortality of more than 80% [3,56]. The incidence rates of PML in SOT are not well defined, but a recent study of HT and LuT recipients estimated 1.29 PML cases per 1000 post-transplant patient years [57]. By comparison, PML was estimated to occur in 2.4 per 1000 patient years in HIV-AIDS patients before availability of combination antiretroviral therapy [58], and 2.1 to almost 10 per 1000 patient years in multiple sclerosis patients treated with natalizumab [59]. The rareness of PML in SOT has impeded the identification of risk factors and the development of a sensitive and specific biomarker for screening comparable to BKPyV viraemia [60–62]. This renders PML typically a clinical diagnosis at an advanced stage of considerable brain damage [3].

Trichodysplasia spinulosa, also called *trichodysplasia* of immunosuppression, *pilomatrix* dysplasia or calcineurin inhibitor-associated folliculodystrophy, is a rare proliferative-cytopathic skin disease with detectable PyV particles in inner root hair follicles of immunocompromised individuals [8]. Clinically, the affected patients develop papules of thickened skin and small hair-like protrusions called *spiculae* most prominently seen in the forehead, nose and ears, together with often circumscribed alopecia (e.g. of the eyebrows). The affected patients belong to all age groups, and frequently have a history of leukemia, chemotherapy or transplantation. The *spiculae* must be distinguished from other digitate hyperkeratoses [63]. There are no reliable data about the rates and risk factors of *Trichodysplasia spinulosa* in SOT, which is likely to occur in less than 1 per 1000 patient years post-transplant, but the disease is probably under-reported. Most cases in SOT have been described in KT recipients, followed by HT and LuT recipients. However, it is unclear whether or not renal failure and KT *per* se predispose to *Trichodysplasia spinulosa*, or if this simply reflects the higher number of immunocompromised KT patients with long-term graft survival and corresponding follow-up.

Merkel cell carcinoma is a rare aggressive cancer that occurs in 0.24 – 0.35 cases per 100 000 patient years [7,64]. The initially often solitary purplish lesion is typically located on sun-exposed skin of elderly white, preferentially immunocompromised individuals, including SOT and HIV patients. The vowels of the alphabet *AEIOU* have been proposed as acronym to summarize characteristic clinical features of Merkel cell carcinoma [65]: *A*symptomatic initially, *E*xpanding rapidly in < 3 months, *I*mmunocompromised, *O*lder than 50 years of age, *U*V-exposed site [65]. MCPyV has been detected in the majority of proven cases and is classified as a class 2A carcinogen [66]. However, the MCPyV genomes are altered due to different chromosomal integration events [66]. This permits expression of early viral gene regions such as the small tumour antigen, but disrupts expression of late viral gene regions, and hence abrogates the (cyto-)lytic viral replication cycle [67,68]. According to the HPyV pathology patterns proposed previously, Merkel cell carcinoma is a non-replicative viral pathology [69]. However, affected patients often have high antibody levels not only to epitopes of the tumour antigens, but also to the capsid proteins [70]. This suggests that significant MCPyV replication must have occurred at an earlier, possibly pre-disease seeding stage. Interestingly, patients with higher antibody titres have been associated with better clinical outcomes, implicating antibody titres as markers of better virus-specific immune responses and outcome [71,72].

Respiratory tract infections have been associated with the detection of KIPyV and WUPyV in respiratory samples, but evidence of proven pathology is lacking. The detection rates of KIPyV and WUPyV range from 1% to 16% and 0.5% to 8%, respectively, in various studies of healthy individuals and patients with respiratory symptoms [6]. No comprehensive data are currently available from SOT, possibly with the exception of LuT recipients [73]. KIPyV and WUPyV were detected in 9.2% and 12.3% of 66 transbronchial biopsies, respectively, with a low tissue viral load around 1200 GEq per 150 000 diploid cell equivalents [74]. The detection of KIPyV and WUPyV was not associated with clinical and histopathological findings, which included acute respiratory insufficiency, interstitial and organizing pneumonia, and acute and chronic rejection. Data from allogeneic haematopoietic stem cell transplant recipients suggest a frequent colonization in asymptomatic patients, but also a possible (co-)factorial role in wheezing and impaired lung function [75]. Similar to allogeneic haematopoietic stem cell transplantation [76], therefore, no recommendation on screening, prevention and treatment of KIPyV and WUPyV can be given at this time for SOT patients. For the remaining HPyVs, no definitive disease association has been identified, including for SOT patients. Thus, HPyV6 and -7 are frequently detected on the skin of healthy and immunocompromised patients, with rates ranging from 10% to more than 30%, HPyV9 has been detected in blood and rarely on skin, and HPyV10 and its closely related variants MWPyV, MXPyV, St-Louis PyV and HPyV12 have been found in faecal samples of individuals with and without gastrointestinal disease [9,77]. Therefore, the role of these HPyVs in SOT is presently unclear and no recommendations for screening for and diagnosis of these HPyVs can be made at this time.

Pre-transplant HPyV Assessment of SOT Donors and Recipients

The recommendations regarding the pre-transplant evaluation of SOT donors and recipients with respect to HPyV serology, NAT and virus-specific T-cell tests are summarized in Table 2. In general, no pre-transplant screening is recommended, except for specific patients that are listed for kidney transplantation after loss of renal function due to PyVAN.

Post-transplant HPyV Assessment and Management of SOT Recipients

The recommendations regarding the post-transplant screening for HPyV in SOT recipients are summarized in Table 3, with the exception of BKPyV in KT recipients. The recommendations for post-transplant treatment presently focus on patients with probable or proven HPyV disease, while presently discouraging general HPyV screening modalities (except for BKPyV in KT recipients), because of insufficient evidence of benefit.

TABLE 2. Recommendations for HPyV-specific *pre-transplant* testing of SOT donors and recipients

Pre-transplant testing of all SOT donors and all SOT recipients for any HPyV DNA in urine, blood or other clinical specimens is not recommended, because current data are not sufficient for guiding organ allocation, risk stratification, immunosuppressive therapy, screening modalities or prophylactic, preemptive or therapeutic intervention pre- or post-transplant (***BIII***)
For SOT recipients listed for kidney transplantation after terminal renal failure due to PyVAN in native or transplanted kidneys, testing of plasma BKPyV should be considered and should be undetectable prior to (re-)transplantation or *at least* have declined by at least >2 $\log_{10}$ GEq/mL compared with documented peak plasma loads (***BIII***)
Pre-transplant HPyV serology of SOT donors or SOT recipients is not recommended, because current data are not sufficient for guiding organ allocation, risk stratification, immunosuppressive therapy, screening modalities, or prophylactic, preemptive or therapeutic intervention pre- or post-transplant (***BIII***)
Pre-transplant testing of HPyV-specific T-cell responses in peripheral blood of SOT donors or SOT recipients is not recommended, because current data are not sufficient for guiding organ allocation, risk stratification, immunosuppressive therapy, screening modalities, or prophylactic, preemptive or therapeutic intervention modalities in SOT recipients (***BIII***)

TABLE 3. Recommendations for *post-transplant* evalution for HPyV in SOT recipients

All SOT recipients or the legal representative in the case of children should be counselled to perform self-examination of skin and lips to identify and report new skin lesions to a health care provider (***BIII***)
All SOT recipients should have an annual skin and lip examination by a qualified healthcare professional with experience in diagnosing proliferative and malignant skin diseases to identify HPyV-associated skin diseases as well as melanoma and non-melanoma skin cancers (***BII***)
Post-transplant routine *screening* of SOT recipients for any HPyV DNA (including JCPyV, MCPyV, TSpyV, KIPyV and WUPyV) in urine, blood or other clinical specimens is not recommended (with the exception of BKPyV in KT recipients), because current data are not sufficient for risk stratification, or for guiding immunosuppressive therapy, screening modalities, or prophylactic, preemptive or therapeutic intervention (***BIII***)
Post-transplant routine *screening* of SOT recipients for any HPyV-specific antibody responses (including BKPyV, JCPyV, MCPyV, TSpyV, KIPyV and WUPyV) is not recommended, because current data are not sufficient for risk stratification, or for guiding immunosuppressive therapy, screening modalities, or prophylactic, preemptive or therapeutic intervention (***BIII***)
For SOT patients with probable HPyV disease, tissue specimens should be obtained for histopathology and specific immunohistochemistry, including staining for HPyV proteins encoded in the early and late viral gene region (e.g. large or small T-antigen, VP1 capsid proteins) to obtain the diagnosis of proven HPyV disease (***BIII***)
Testing tissue specimens for HPyV DNA by NAT cannot substitute for immunohistochemistry, but may be considered as an adjunct tool for diagnosis, if immunohistochemistry is not possible (***BIII***)
HPyV DNA in tissue specimens should be reported as genome equivalents per diploid cells to permit semi-quantitative comparison of the results with those from unaffected tissues (***BIII***)
In the absence of specific histopathology, the presence may be determined by HPyV genome alterations that have been consistently linked to, and may independently support, the diagnosis of a HPyV disease (e.g. rearrangements of the non-coding control region, point mutations and truncations of the early and late viral gene region) (***BIII***)

KT recipients

Screening. Post-transplant screening is currently only recommended for BKPyV in KT patients as summarized in Table 4. International guidelines recommend a minimal screening for

TABLE 4. Recommendations for *post-transplant* BKPyV diagnostic testing of kidney transplant recipients

All kidney transplant recipients should be regularly screened for BKPyV replication in urine (viruria) or plasma (viraemia) to identify patients at increased risk of PyVAN (***AII***)
Screening plasma for BKPyV loads is recommended monthly in the first 6 months post-transplant, followed by 3-monthly screening until 2 years post-transplant (***AII***)
Testing urine for significant BKPyV replication may be an alternative modality by identifying 'decoy cells', PyV aggregates, BKPyV VP1 mRNA or significant urine BKPyV DNA loads of $\geq$7 log $_{10}$ GEq/mL, but positive cases should be followed-up by quantifying plasma BKPyV loads (***BII***)
Testing plasma for BKPyV loads is recommended to guide therapeutic interventions for kidney transplant patients with probable (presumptive) or proven PyVAN (***AII***)
Testing plasma for BKPyV loads is recommended for KT patients having an unexplained serum creatinine rise, after treatment of acute rejection, or undergoing protocol biopsies (***BIII***)
In the absence of an international calibrator, plasma BKPyV load data should be compared by the same NAT assay, preferably in the same laboratory, which is operated according to quality assurance programmes and certified for transplantation diagnostics (***BIII***)

BKPyV replication of *at least* once every 3 months in the first 2 years post-transplant, then annually thereafter for 5 years, and in the event of an unexplained serum creatinine rise or after treatment for acute rejection [15,16,19]. However, it is advisable to adapt the screening algorithm according to centre-specific characteristics (such as very low or very high PyVAN incidence, paediatric cohorts and sensitized recipients). Specifically, as *de novo* BKPyV replication preferentially occurs within the first 6 months post-transplant, and most cases of PyVAN occur in the first year post-transplant, intensified screening by monthly sampling in the first 6 months is recommended, followed by decreasing frequency thereafter [15,78]. BKPyV viraemia has a positive predictive value of 30–50% for proven PyVAN [79], that increases to more than 90% in the presence of very high plasma BKPyV loads, appearance of genomic BKPyV variants with rearrangements in the non-coding control regions [80], or when renal dysfunction is present.

The sensitivity of PyVAN histology may be increased by adequately sized biopsies, use of larger biopsy needles (e.g. gauge 18) and the presence of renal medulla. Nevertheless, kidney allograft biopsies may be falsely-negative at an estimated rate of 10–30% in early stages of PyVAN [81]. Hence, kidney recipients with sustained plasma BKPyV loads above 1000 GEq/mL are at risk and have been diagnosed as having 'presumptive' PyVAN, when increasing to more than 4 $\log_{10}$ Geq/mL [15,19]. The shorter window period observed between BKPyV viraemia and PyVAN has suggested monthly monitoring as the preferred strategy in some centres, particularly those screening plasma directly. Anecdotal cases of kidney recipients with histologically proven PyVAN found negative for BKPyV viraemia have been reported [82]. In these cases, methodological issues need to be ruled out [83,84] as well as the presence of a rare case of JC-PyVAN, which typically shows low or undetectable plasma JCPyV loads despite persisting high urine JCPyV loads >7 log GEq/mL [21,55]. In many cases, JCPyV-mediated PyVAN is cleared after reducing immunosuppression [21]. No universal screening for JCPyV can be recommended given the rarity of this condition, but persisting high urine JCPyV loads in the absence of BKPyV loads may raise suspicion [15,21].

Screening for BKPyV replication can also be performed by testing urine for high-level BKPyV viruria, which precedes viraemia and nephropathy by 4 to 12 weeks, and can effectively rule out PyVAN due its high negative predictive value [24]. Urine screening is non-invasive and relatively inexpensive when searching for 'decoy cells' by conventional cytology. However, the low positive predictive value for PyVAN and unreliable clearance kinetics limit the use of viruria as a tool to guide therapeutic intervention. A higher positive predictive value has been reported when urine BKPyV was tested by the VP1 mRNA transcripts [85] or through electron microscopy evaluation of three-dimensional viral aggregates [86]. However, the latter approaches require expertise and EM instrumentation not widely available, and. Moreover, independent prospective data are not available to guide recommendations on their clinical utility, particularly regarding early, preemptive management. Thus, a positive urine result should be followed-up by testing for plasma BKPyV loads to identify patients with presumptive PyVAN and follow their response to intervention.

Prophylaxis and treatment of probable and proven PyVAN. Interventions for BKPyV replication and PyVAN potentially encompass prophylactic, preemptive and therapeutic modalities. There are no BKPyV-specific antiviral drugs for prophylaxis or treatment, but the use of fluoroquinolones such as ciprofloxacin and levofloxacin after kidney transplantation has been associated with a reduced frequency of BKPyV replication in limited retrospective studies and case series [87–89]. The use of mTOR inhibitors has been associated with reduced BKPyV events in registry studies and in retrospective analyses, but a general recommendation must be balanced against the overall graft function and outcome [52,90,91]. However, the data are currently insufficient to assess efficacy, side-effects including bacterial resistance and *Clostridium difficile* colitis, costs and the resulting cost–benefit ratio before specific recommendations for or against their use can be made (Table 5). Intravenous immunoglobulin preparations have been shown to contain BKPyV-specific antibodies that increase in KT patients with BKPyV replication and PyVAN [92–95], but their role in prophylaxis and treatment is currently unresolved [96,97]. Early detection of BKPyV replication and disease seems to be important for improved outcomes. Prospective studies show that progression to PyVAN can be effectively

TABLE 5. Recommendations for prophylaxis and treatment of probable and proven polyomavirus-associated nephropathy in kidney transplant recipients

Based on the presently available data, administration of fluoroquinolones, mTOR inhibitors and intravenous immunoglobulins is not recommended as prophylaxis against BKPyV replication and disease in kidney transplant patients (***CIII***)

In kidney recipients with confirmed (sustained) plasma BKPyV loads or presumptive PyVAN, maintenance immunosuppression should promptly be reduced in a step-wise fashion unless other competing risks are imminent (***AII***)

In kidney transplant patients with proven PyVAN, maintenance immunosuppression should promptly be reduced in a step-wise fashion unless other competing risks are imminent (***AII***)

Presently, a specific immunosuppression minimization strategy can not be recommended, but may consist of reducing the calcineurin inhibitor, and/or reducing and/or discontinuing the antiproliferative drug, switching immunosuppressive drugs, or a combination thereof (***CIII***)

Kidney transplant patients undergoing minimization of maintenance immunosuppression should be followed by measuring at least once- to bi-weekly serum creatinine concentration to estimate renal allograft function and plasma BKPyV loads to identify clearance of viraemia (***BII***)

Kidney allograft biopsy is not routinely recommended in patients in whom immunosuppression has been reduced for treating probable or proven PyVAN and renal allograft function is stable (***AII***)

Kidney allograft biopy is recommended when renal allograft function significantly decreases from baseline to guide further treatment decisions regarding immunosuppression minimization or treatment of acute rejection (***AII***)

Based on the currently available data, administration of cidofovir, leflunomide, fluoroquinolones, mTOR inhibitors or intravenous immunoglobulins is not recommended as sole or adjunct treatment of kidney transplant recipients with probable or proven PyVAN (***BIII***)

and safely hindered using a preemptive reduction in immunosuppression [50,98–101]. However, supporting data do not originate from randomized treatment trials.

Two different immunosuppression reduction protocols, or their combination, have been proposed to treat confirmed (sustained) BKPyV viraemia and 'presumptive' PyVAN (Table 6). One approach first considers reducing calcineurin inhibitors (CNI) by 25–50% in one or two steps [36], followed by reduction/discontinuation of the anti-metabolite at a later time [98–100], while the other reduces/discontinues the anti-metabolite, and only subsequently the CNI [50]. Alternatively, the concomitant reduction of both CNI and anti-metabolite has been reported [100]. All strategies appear safe in the short term at 1 year of follow-up, showing 4–14% subsequent acute rejections, all of which were responsive to steroid therapy. Efficacy was demonstrated in both adult and paediatric kidney recipients by clearance of plasma BKPyV loads within weeks to a few months after intervention (Table 6). However, despite the preemptive timing of reducing immunosuppression, progression to histologically proven PyVAN cannot be completely prevented, as demonstrated by protocol biopsies [78,99,100]. These cases were typically associated with higher plasma BKPyV loads and required multiple steps of immunosuppression reduction, and longer times until clearance of viraemia [99]. Although standard operating procedures should be defined in each transplant centre, the decisions should integrate individual patient factors and CNI trough levels. The effect of immunosuppression minimization on long-term outcome has been examined in one study [101], but remains to be assessed for other approaches.

Minimization of immunosuppression, according to the principles described for the preemptive strategy, is also the mainstay of treatment for proven PyVAN. In the latter case, a more aggressive approach to immunosuppression reduction may be required, targeting drug levels below the lower end of the therapeutic range [15,16]. Alternatively to immunosuppression reduction, a switch from tacrolimus to low-dose cyclosporine A and from mycophenolic acid to an mTOR inhibitor, preferentially with simultaneous CNI reduction, has also been employed in small case series with some success. Other treatment attempts such as cidofovir, leflunomide, intravenous immunoglobulins or fluoroquinolones, have been variably employed. So far, no randomized trial has demonstrated the superior efficacy

TABLE 6. Treatment by reduced immunosuppression in kidney transplant patients with presumptive or proven polyoma-associated nephropathy

	Brennan *et al.* 2005[50]	Ginevri *et al.* 2007[98]	Schaub *et al.* 2010[99]	Sood *et al.* 2012[100]
Patients	Adult (n = 200)	Paediatric (n = 52)	Adult (n = 203)	Adult (n = 240)
BKPyVviraemia n (%)	23 (12)	13 (21)	38 (19)	65 (27) 28 (12.5) with plasma BKPyV load >4log Geq/mL
Proven BK-PyVAN n (%)	None (no protocol allograft biopsy performed)	None (no protocol allograft biopsy performed)	13 (34%) of 38 viraemic patients with allograft biopsy (including protocol allograft biopsy)	5 (21%) of 23 viraemic patients with allograft biopsy (no protocol allograft biopsy)
Intervention	Step 1: discontinue azathioprine or MMF Step 2: reduce CNI	Step 1: reduce CNI Step 2: reduce or discontinue MMF	Step 1: reduce CNI Step 2: reduce or discontinue MMF	Step 1: reduce CNI and reduce MMF Step 2: repeat
Outcome at 1 year				
Clearance of viraemia, %	**95**	**100**	**92**	**80**
Time to clearance (range)	1.8 months (7–213)	2 months (1–8)	4.4 months (1–22)	6–12 months
Acute rejection, n (%)	**1 (4.3)**	**0 (0)**	**3 (8.6)**	**4 (14.3)**
Graft loss	None	None	None	1

Significance of bold is just general emphasis as these are the key results.

of any combined approach over immunosuppression reduction alone [102].

The use of mTOR inhibitors has been linked to a reduced rate of BKPyV events in large registry studies and in single-centre observations [103]. Prospective studies are underway evaluating the effect of mTOR inhibitors on BKPyV replication as a secondary endpoint. Virus transmission from the BKPyV seropositive graft to a seronegative recipient could theoretically be prevented or limited by administration of specific intravenous immunoglobulins. However, no specific immunoglobulin or BKPyV vaccine has been developed. A role of fluoroquinolones was suggested by *in vitro* studies [104], but retrospective clinical studies showed variable results [87,89]. Randomized, prospective studies are ongoing; these may clarify the role of quinolones in preventing BKPyV viraemia and PyVAN [88].

Histopathology. The recommendations regarding the histological diagnosis of PyVAN in SOT recipients are summarized in Table 7. As discussed, the focal nature of PyVAN may result in sampling errors in 10–30% of cases [81]. Hence, a negative biopsy does not rule out PyVAN, particularly in the early stages. Sampling of medulla reduces the chance of a false-negative biopsy. For immunohistochemistry, a cross-reacting monoclonal antibody directed against the large T-antigen of the Simian virus 40 (clone PAb 416, Calbiochem) is commonly used. There is considerable inter-laboratory variation in staining intensity and assessment of percentage of infected cells, but the binary classification of biopsies into virus-positive and negative is fairly reliable.

Standardized assessment and reporting is suggested to improve consistent biopsy readings across multiple institutions. Classification of PyVAN into categories PyVAN-A, -B and -C within the same institution is reasonably reproducible ($\kappa = 0.47$) [105]. As most biopsies belong to class B, reporting of subgroups B1, B2 and B3 defined by the increasing percentage of biopsy area affected should be considered (Table 8). More recently, the 2009 Banff conference formulated a working proposal wherein stage A and B were defined by the extent of BKPyV-mediated cell injury. In this system, an identical stage can be assigned to biopsies that differ markedly in the degree of inflammation, and hence differ in prognosis [106]. However, inflammation and extent of fibrosis and tubular atrophy at diagnosis may be the most important predictors of a poor outcome.

Intimal arteritis, glomerulitis and peritubular capillary C4d deposits are histologic criteria that help to differentiate acute rejection from PyVAN. Tubulitis is not a reliable discriminating parameter, even if present away from areas of viral cytopathic effect. MHC class II upregulation by the tubular epithelium is no longer considered a marker specific for rejection as it also occurs in the context of viral replication [107]. Occasionally, other viruses such as JCPyV, cytomegalovirus and adenovirus may result in viral inclusions. Specific immunohistochemistry for the respective viral antigens is needed to confirm these other diagnoses.

The incidence of acute T-cell mediated rejection (ACR) after reduction of immunosuppression is approximately 10%. Kidney biopsies are difficult to interpret during this phase. Morphological criteria for resolving PyVAN and T-cell-mediated rejection overlap, and the differential diagnosis is facilitated by careful clinical correlation and attention to serial trends in serum creatinine and viral loads in plasma and then urine [15,107]. Analyzing T-cell specificity by next generation sequencing of T-cell receptor usage might provide a future tool in the differential diagnosis [108]. In general, response to steroids is seen in only a third to a half of biopsies that otherwise satisfy Banff criteria for acute rejection [109,110]. Clinical management in this setting is uncertain until better diagnostic criteria are developed and effective anti-BKPyV drugs become available.

TABLE 7. Histological diagnosis of polyomavirus-associated nephropathy in kidney and non-kidney SOT recipients

For a histological diagnosis, a minimum of two kidney biopsy cores, preferably containing medulla tissues, is recommended in order to reduce the rate of false-negative results (***BII***)
The diagnosis of proven PyVAN requires compatible cytopathic changes in renal tubular cells and demonstration of PyV replication by immunohistochemistry or *in-situ* hybridization (***AII***)
The histological findings of PyVAN should be semi-quantitatively assessed using proposed criteria to systematically capture the extent of viral, inflammatory and fibrotic changes as markers of prognosis (***BII***)
JCPyV should be considered as aetiological agent if biopsies show histological signs of intranuclear inclusions, interstitial infiltrates and tubulitis and SV40 large T-antigen-positive immunohistochemistry in renal tissue, but undetectable viremia (***BIII***)
Other viral agents such as human adenovirus and cytomegalovirus should be considered if biopsies show histological signs of intranuclear inclusions, interstitial infiltrates and tubulitis, but absence of confirmatory immunohistochemistry in renal tissue (***BIII***)

Non-kidney SOT recipients

The clinical significance of BKPyV replication in non-kidney SOT recipients is less clear. In a prevalence study comparing 156 consecutive KT, HT or LiT recipients, BKPyV viruria was found in 26.5%, 25.5% and 7.8%, respectively, of the patients. BKPyV viraemia was found in 12.2% of KT recipients and 7% of HT recipients, but not in LiT recipients, and no disease attributed to BKPyV was seen [111]. Cases of PyVAN have been observed in non-kidney SOT recipients, albeit rarely. In a recent review, nine non-kidney transplant patients with PyVAN were described (six HT, two LuT and one PT). Five patients received triple immunosuppression consisting of tacrolimus, mycophenolate and prednisone. PyVAN was

TABLE 8. Histological patterns of polyomavirus-associated nephropathy

Pattern	Description	Extent of biopsy core	Graft function	Risk of graft loss
PyVAN-A				
Viral cytopathic changes	Mild	Variable (typically <25%)	Mostly baseline	<10%
Interstitial inflammation	Minimal	≤10%		
Tubular atrophy	Minimal	≤10%		
Interstitial fibrosis	Minimal	≤10%		
PyVAN-B *				
Viral cytopathic changes	Variable	Variable (often 11 – >50%)	Mostly impaired	50%
Interstitial inflammation	Significant	11 – >50%		
Tubular atrophy	Mild/moderate	<50%		
Interstitial fibrosis	Mild/moderate	<50%		
PyVAN-B1				
Interstitial inflammation	Moderate	11–25%	Slightly above baseline	25%
PyVAN-B2				
Interstitial inflammation	Significant	26–50%	Significantly impaired	50%
PyVAN-B3				
Interstitial inflammation	Extensive	>50%	Significantly impaired	75%
PyVAN-C				
Viral cytopathic changes	Variable	Variable	Significantly impaired progressive failure	>80%
Interstitial inflammation	Variable	Variable		
Tubular atrophy	Extensive	>50%		
Interstitial fibrosis	Extensive	>50%		

*Subclassification of PyVAN-B into categories B1, B2 and B3 was initially proposed by Drachenberg *et al* [81]. Using both inflammation and tubular atrophy, biopsies with >50% involvement were designated B3. However, tubular atrophy >50% usually correlates with interstitial fibrosis >50%, which is used to define PyVAN-C. For simplicity, it is suggested that subclassification PyVAN-B be based entirely on inflammation, which is an important and independent predictor of outcome [106].

diagnosed at a median of 21 months after transplantation (range 13–25) and five patients eventually required dialysis [112]. Where administered, there was little response to intravenous cidofovir. The poorer prognosis of PyVAN in non-kidney transplant recipients may be related to the fact that the diagnosis was made at a late stage of significant functional impairment, the difficulty in sufficiently reducing immunosuppression without causing life-threatening rejections, and a delayed recovery of BKPyV-specific immunity. Therefore, in non-kidney SOT patients with declining renal function, testing for BKPyV viremia should be considered early in the diagnostic work-up to identify patients with presumtpive PyVAN.

HT recipients. Studies in HT recipients report rates of BKPyV viruria and viraemia of 11–25% and 7–21%, respectively [113–115]. BKPyV viraemia is an early event after HT as 83% occurred during the first 3 months post-transplant. BKPyV replication in urine and blood was associated with a higher rate of mild renal failure ($p = 0.018$). However, only isolated cases of proven PyVAN have been reported in HT recipients [116–126]. Accordingly, most centres do not routinely screen for BKPyV infection in HT recipients.

LuT recipients. A prospective longitudinal study performed in 50 LuT recipients over the course of 17 months detected BKPyV, JCPyV or SV40 in 31/50 (62%) of the patients' urine samples at least once [127]. BKPyV was present in 16/50 (32%) of the patients but all blood samples were negative. There was no significant association between acute rejection and patients who shed polyomavirus but patients with shedding had a worse survival. However, PyVAN leading to terminal renal failure and haemorrhagic cystitis have been reported in a small number of adult and paediatric LuT recipients [128–130]. PyVAN was detected in a 67-year-old female recipient 60 months after LuT [112]. Recently, a case of BKPyV-associated cancer has been reported in a paediatric LuT recipient who first was diagnosed with PyVAN at 2 years post-transplant leading to end-stage renal failure and then was diagnosed with *ductus Bellini* carcinoma of the native kidney [131].

LiT recipients. BKPyV viruria was detected in 7.8–21% and viraemia ranged from 4% to 18% of LiT recipients [111,114,115,132–135]. BKPyV viraemia occurred mostly in the first 3 months and was more common in patients with recent rejection episodes. No relationship between BKPyV replication and maintenance immunosuppression was observed. In one study, three patients with sustained BKPyV viraemia developed renal insufficiency, but PyVAN was not histologically confirmed. One of the patients died of multi-organ failure with non-identified viral inclusions in the liver biopsy and a persistent and extremely high BKPyV viral load [132].

Treatment of other HPyV disease in SOT recipients

Progressive multifocal leukoencephalopathy (PML) is clinically suspected when facing new-onset progressing CNS deficits (such as motor weakness, speech abnormalities, cognitive deficits, visual field deficits and ataxia) and compatible features on MRI studies (such as hypointense T1-weighted lesions that appear hyperintense on T2-weighted lesions or fluid-attenuated inversion recovery (FLAIR) sequences) [3,136]. Typically, the affected persons have abnormalities of the immune system,

TABLE 9. Recommendations for the management of progressive multifocal leukoencephalopathy (PML) and other JCPyV-associated diseases in SOT recipients

In SOT recipients with the diagnosis of possible PML (new onset CNS deficits, T2-hyperintense, T1-hypointense, preferably subcortical lesions), CSF should be examined for JCPyV DNA as virological confirmation (i.e. probable PML) (***AIII***)
In SOT recipients with possible PML and negative JCPyV DNA results in CSF, stereotactic biopsy of affected brain lesions should be considered if clinically indicated to histologically confirm PML (proven PML) or to obtain another diagnosis (***AIII***)
In SOT recipients with probable or proven PML, maintenance immunosuppression should be reduced promptly to a lower limit that permits allograft function and rapidly enables the restoration of JCPyV-specific immune control (***BIII***)
In kidney and pancreas transplant recipients with probable or proven PML, discontinuation of maintenance immunosuppression and return to functional organ substitution should be considered to rapidly enable mounting of JCPyV-specific immune control (***BIII***)
In SOT patients with JCPyV-associated granule cell neuronopathy or encephalopathy, reducing maintenance immunosuppression similar to the treatment of PML should be considered (***BIII***)
No recommendations can be made regarding the treatment of SOT recipients with probable or proven PML using serotonin-uptake inhibitors of the type mirtazapine, intravenous cidofovir, high-dose intravenous immunoglobulins, leflunomide or brincidofovir (CMX001) (***CIII***)

but the disease has been detected in presumably immunocompetent individuals. The strength of the diagnosis is reflected in the diagnostic criteria: probable PML is virologically confirmed when JCPyV DNA is detected in the CSF, whereas proven PML requires histological confirmation of demyelination and the detection of JCPyV replication by immunohistochemistry or *in situ* hybridization in brain tissues (Table 9).

There is no established antiviral treatment for PML and mounting a JCPyV-specific immune response to (re-)gain control over JCPyV replication in the CNS is the key objective [137,138]. In HIV-AIDS patients with PML or in multiple sclerosis patients with PML after natalizumab use, this can be achieved in part by starting combination antiretroviral therapy or discontinuing and removing natalizumab by plasma exchange, respectively. However, in SOT recipients, treatment is limited by acute rejection and subsequent graft loss. For KT or PT patients, decreased or discontinued immunosuppressive medication, which leads to return to dialysis or insulin substitution, is a potentially viable option [139], whereas this is not the case for HT, LuT and LiT. Recently, a case of PML in an HSCT patient was treated with donor-derived JCPyV-specific T cells [38], suggesting the possibility that immune control may be enhanced by autologous T-cell therapy in SOT patients, most likely still requiring reduced immunosuppression.

Currently, there are no data supporting recommendations about treatment of PML in SOT patients. Whenever possible, therefore, SOT patients with probable or proven PML should be enrolled in appropriate clinical treatment trials, and/or clinical experts should be consulted in individual cases. Antiviral treatments are desirable because they potentially should limit the cytopathic damage caused by JCPyV replication and thereby extend the window for immune recovery [3]. Because of the potential (co-)role of serotonin receptors in JCPyV infection, some experts recommend treatment with mirtazapine, starting with 15 mg per day then increasing to the highest possible dose of, for example, 45 mg per day [140–142]. However, data from clinical trials are lacking. Mefloquine is not effective in clinical trials and has significant side-effects, including in the CNS, which argue against its clinical use [143]. Cytarabine does not cross the intact blood-brain barrier and has not been effective in a randomized clinical trial of PML in HIV-AIDS despite intrathecal administration [144].

Similarly, intravenous cidofovir has not been shown to be effective in retrospective and prospective studies of HIV-AIDS patients with PML over antiretroviral therapy alone [145,146]. However, intravenous cidofovir has been studied as prophylaxis and treatment for cytomegalovirus retinitis, with some evidence of efficacy at 5 mg/kg body weight in studies GS-93-105, -106 and -107 as discussed [147–149]. Cidofovir is unlikely to cross the intact blood-brain barrier efficiently without organic anion transporters. These are present in the eye and the renal tubulus, contributing to uveitis/iritis and renal toxicity. Other side-effects include neutropenia, metabolic acidosis and pancreatitis [148]. However, the blood-brain barrier may be locally impaired in SOT recipients, especially when there are signs of contrast-enhancement on MRI. CMX001 (recently called brincidofovir), an oral lipid conjugate of cidofovir, has shown 400-fold higher *in vitro* efficacy against JCPyV [150], and reaches higher intrathecal concentration in animal models. Recent clinical studies suggest efficacy against cytomegalovirus replication in allogeneic HSCT recipients when dosed at 100 mg per os twice weekly, without renal toxicity, but with diarrhoea as a major adverse event [151]. However, there are as yet no clinical trials demonstrating its potential clinical efficacy in PML. Therefore, the current management of probable or proven PML in SOT patients remains a clinical challenge (Table 9). Similar considerations must also be applied to patients with other JCPyV-associated diseases such as granule cell neuronopathy, encephalopathy or nephropathy [3].

Merkel cell carcinoma is diagnosed on clinical grounds and the key predictor of outcome is invasiveness and metastasis into the local lymph node and disseminated disease [7,152,153]. Treatment is stage dependent and requires the assessment of whether or not the disease is local or has metastasized in regional lymph nodes as assessed macroscopically, microscopically by sentinel lymph node biopsy and/or by [18] F-PET computed tomography [153], or has disseminated [7]. Accordingly, stage-adapted treatment escalation is recommended by the American Joint Committee on Cancer (AJCC) [154]. Based on the TMN staging, surgical resection of localized tumours is combined with radiotherapy, or with

additional radiotherapy of the regional lymph nodes for larger tumours or definite lymph node involvement, whereas chemotherapy is the key approach for metastatic Merkel cell carcinoma. Overall, the experience of treating SOT recipients with Merkel cell carcinoma is limited to delineate specific recommendations. Expert centres dealing with skin malignancies should be contacted early in the process of diagnosis and staging and referral of patients considered. Improving the overall immune function is likely to play a role in the overall management, but the feasibility and efficacy of this approach is not documented and highly dependent on the underlying organ transplant. There is no role of antivirals in the treatment of this non-replicative disease, but interferon-a treatment for non-transplanted patients and more specific drugs aimed at the viral oncogene–host cell interaction may be future developments.

Trichodysplasia spinulosa is clinically suspected because of its characteristic skin features [8]. Proven diagnosis requires skin biopsies for histopathology and immunohistochemistry, which is, however, not frequently performed when balancing the moderate invasiveness against the diagnostic certainty in clear cases. Given the broader differential diagnosis of digitate hyperkeratotic diseases [63], collection of the protruding *spiculae* for TSPyV NAT is a non-invasive alternative. However, as TSPyV has been detected in healthy persons, the role of qualitative TSPyV NAT in diagnosis is not resolved. Treatment consists of improving immune function, which in SOT recipients consists of reducing maintenance immunosuppression, ideally without precipitating acute rejection episodes [8]. There is no evidence suggesting the preferential reduction of CNIs over the antiproliferative agent, but given the importance of T-cell activation, it seems plausible that reducing the CNI has a critical role in a step-wise approach. Topical antivirals (i.e. cidofovir 1–3% cream) have been successfully combined with reduced immunosuppression, without detrimental local or systemic effects such as renal, haematopoietic or ocular toxicity. Presumably, the TSPyV actively recruits the host cell DNA polymerase in the infected cells for effective viral replication. Thereby, infected cells and the viral effects are presumably becoming more sensitive than non-infected cells to this high local concentration of cidofovir. Thus, there is currently little evidence to recommend specific approaches for the treatment of *Trichodysplasia spinulosa*, but it seems reasonable to consult an expert in dermatological diseases in SOT.

Conclusions

Human polyomaviruses are a growing challenge in immunocompromised patients in view of the increasing number of different virus species and their diverse disease potential, including in SOT recipients. The distinction between infection, replication and disease is not only important for clinical studies, but also helps to improve diagnosis and management of probable and proven HPyV disease in clinical practice. The quality of the clinical evidence and the strength of most recommendations are presently limited, but are expected to be improved in the coming years.

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